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Measurement of nutritional status in simulated microgravity by bioelectrical impedance spectroscopy

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Bartok, Cynthia, Richard L. Atkinson, and Dale A. Schoeller. Measurement of nutritional status in simulated microgravity by bioelectrical impedance spectroscopy. *J Appl Physiol* 95: 225–232, 2003. First published January 31, 2003; 10.1152/japplphysiol.00721.2002.—The potential of bioelectrical impedance spectroscopy (BIS) for assessing nutritional status in spaceflight was tested in two head-down-tilt bedrest studies. BIS-predicted extracellular water (ECW), intracellular water (ICW), and total body water (TBW) measured using knee-elbow electrode placement were compared with deuterium and bromide dilution (DIL) volumes in healthy, 19- to 45-yr-old subjects. BIS was accurate during 44 h of head-down tilt with mean differences (BIS − DIL) of 0–0.1 kg for ECW, 0.3–0.5 for ICW, and 0.4–0.6 kg for TBW (n = 28). At 44 h, BIS followed the within-individual change in body water compartments with a relative prediction error (standard error of the estimate/baseline volume) of 2.0–3.6% of water space. In the second study, BIS did not detect an acute decrease (−1.41 ± 0.91 kg) in ICW secondary to 48 h of a protein-free, 800 kcal/day diet (n = 18). BIS's insensitivity to ICW losses may be because they were predominantly (65%) localized to the trunk and/or because there was a general failure of BIS to measure ICW independently of ECW and TBW. BIS may have potential for measuring nutritional status during spaceflight, but its limitations in precision and insensitivity to acute ICW changes warrant further validation studies.

total body water; extracellular water; intracellular water; head-down tilt; body composition

LONG-TERM SPACEFLIGHT HAS the potential to produce alterations in bone, water, muscle, and fat mass components. The first body composition change is a rapid loss in extracellular water (ECW) that occurs 1–2 days after entering space, including a 10% loss in plasma volume (3). This is likely due to reductions in fluid and food intake (7). As the duration of spaceflight increases, a state of negative nitrogen balance and loss of muscle mass occur (28). In addition, body fat stores may decline significantly with long-term space missions. These body tissue losses can limit strength, performance of duties, and an astronaut's ability to complete emergency procedures should the need for such procedures arise.

Despite the importance of monitoring body composition during flight, these measurements have not been made because of limitations imposed by spaceflight. Most of the equipment needed to measure body composition takes up too much volume (e.g., that required for hydrostatic weighing), or the methods require Earth-based laboratory analysis (e.g., isotope dilution). One method that would meet the practical requirements for spaceflight and might potentially have the sensitivity to detect body composition changes is bioelectrical impedance analysis (BIA). This technique was originally developed by Hoffer et al. (14) and is based on the principle that different body tissues have different electrical properties. BIA employs a single-frequency (50-kHz), alternating-current signal that is applied across the body. The ionic media in fat-free mass conduct the current, while the insulating properties of body fat provide resistance to current flow. The length^2/resistance of the body is highly correlated to total body water (TBW) and fat-free mass (15, 18). The validity of the technique has been well documented (6, 15).

One important limitation of the single-frequency (50-kHz) bioelectrical impedance model is that the primary current path is extracellular. Thus any disruption in the ratio of ECW to intracellular water (ICW) increases the error of body composition prediction (2, 11). This clearly poses a problem for analysis during spaceflight, in which ECW is redistributed from the periphery to the trunk and substantial extracellular losses occur (3). The ability to differentiate between the loss of ECW due to fluid shifts and the loss of ICW due to muscle atrophy is vital to the usefulness of body composition data in spaceflights (17).

The development of multifrequency bioelectrical impedance spectroscopy (BIS) machines provides hope that in-flight body composition measurements can be made. Whereas BIA employs a single frequency, BIS measures the resistance and reactance at various frequencies. At low frequencies, cell membranes block the flow of current through the intracellular space. Thus the path of the electrical current is predominantly through ECW. As the frequency increases, cells begin to act as capacitors and the ICW becomes part of the current path. The ability of BIS to exploit the capacitive nature of intact human cells allows the machine to discern ICW and ECW (4, 29). This information is vital to determining whether changes in body composition

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are related to changes in plasma volume or cell volume, which has ramifications for the treatments prescribed to astronauts. In addition, a demonstration that BIS can measure body water compartments under conditions where the normal distribution of ICW and ECW is altered might extend the use of bioelectrical techniques to clinical situations such as protein malnutrition and cardiovascular insufficiency.

The goal of this study is to validate BIS as a form of body composition analysis for simulated spaceflight. We designed two ground-based bed-rest studies to determine the accuracy and precision of BIS-predicted water compartmentalization under conditions of adequate nutrition and short-term undernutrition. In *study 1*, where nutritional intake was adequate, we expected that the majority of TBW losses would be from the ECW (7). In *study 2*, where nutritional intake was suboptimal, we expected losses of ECW and ICW. We hypothesized that BIS-predicted TBW, ECW, and ICW volumes would be accurate and precise under both conditions.

METHODS

Study 1 protocol. On *day 1*, subjects reported to the University of Wisconsin General Clinical Research Center by 8 AM after an overnight fast. Weight and height were measured. TBW, ECW, and ICW were assessed by dilution methods. In addition, multifrequency BIS was measured using proximal electrode placement (25). Electrode placement was marked with waterproof ink. At approximately 1 PM, subjects were given a liquid meal (Ensure). They received a volume of supplement that provided approximately one-third of their estimated daily energy needs. After this ambulatory period, the subjects assumed the 6° head-down tilt (HDT) position for the duration of the study (~44 h). At 4 h after the subjects assumed HDT, body water volumes and BIS were measured again. Subjects received a low-sodium (2,032 ± 288 mg), adequate-energy (1,734 ± 284 kcal, 66 ± 11 g protein, 244 ± 41 g carbohydrate, 57 ± 9 g fat) diet. Subjects consumed ≥30 ml/kg fluids on each day of the study. Caffeine and alcohol were not allowed.

On *day 2*, subjects remained in the HDT position. In the morning, body water volumes and bioelectrical impedance measurements were repeated (20 h in HDT). After the measurements were completed, subjects received the study diet.

On *day 3*, subjects remained in the HDT position. In the morning, body water volumes and bioelectrical impedance measurements were repeated (44 h in HDT). After the measurements were completed, subjects were given regular food and rehydration fluids. Subjects were discharged when they demonstrated orthostatic tolerance.

Study 2 protocol. Subjects reported to the University of Wisconsin General Clinical Research Center by 6 PM. Weight and height were measured. At 10 PM, subjects assumed the 6° HDT position in bed, which continued for the remainder of the study. Subjects received a low-sodium (1,080 ± 226 mg), adequate-energy (563 ± 95 kcal, 23 ± 4.1 g protein, 80 ± 14 g carbohydrate, 19 ± 3.7 g fat) evening meal.

On *day 2*, subjects completed body water analysis as specified in *study 1* (12 h HDT). BIS measurements included the standard whole body proximal measurements as described for *study 1* as well as segmental analysis (22) using the proximal electrode placement. Electrode placement was marked with waterproof ink. After the measurements were completed, subjects began receiving the hypocaloric research diet designed to induce the negative nitrogen balance and glycogen depletion necessary to decrease intracellular volume. This clear liquid diet provided 800 kcal/day (191 g carbohydrate, 4 g protein, 2 g fat) and 1,080 mg sodium. Subjects consumed ≥30 ml/kg fluids on each day of the study. A multivitamin/mineral pill was given so that micronutrient requirements were met. This diet continued for the duration of the study.

On *day 3*, subjects remained in HDT and received the study diet.

On *day 4*, subjects remained in HDT. In the morning, body water volumes and bioelectrical impedance measurements as described for *day 2* were repeated (60 h HDT, 48 h underfeeding). After all study procedures (72 h HDT), subjects were given regular food and rehydration fluids. Subjects were discharged when they demonstrated orthostatic tolerance.

Subjects. A description of the sample of healthy women and men participating in the studies is provided in Table 1. The subject pool included 39 Caucasians, 6 Hispanics, and 1 African-American. Subjects were of normal weight, were weight stable in the previous month (<2-kg change), and were not taking any medications known to affect body composition. Subjects ranged in age from 19 to 45 yr and had no medical contraindications to the protocol. Women participated during the 7 days between the end of menses (*day 7* of cycle) and before ovulation (*day 14*). Women on oral contraceptive pills were not excluded from participation. All subjects gave written informed consent before participation. The institutional review board of the University of Wisconsin approved the protocol design and all procedures.

Body water assessment. Analysis of TBW, ECW, and ICW was completed using tracer-dilution methods. For all assessments, baseline blood samples were collected for deuterium and bromide analysis. Subjects were then dosed with 10 mg/kg NaBr and 40 g of 10 atom% deuterium solution. Blood samples were collected 4 h after dosing. All urine output during the 4-h tracer equilibration time was collected and pooled so that urinary losses of deuterium and bromide could be directly measured.

TBW was calculated from deuterium dilution as described by Schoeller (26). Deuterium enrichment of plasma and urine samples was quantified using isotope ratio mass spectrometry. Corrections were made for lost dosage of deuterium in

Table 1. *Subject characteristics*

	Study 1		*Study 2*	
	Men (n = 14)	Women (n = 14)	Men (n = 10)	Women (n = 8)
Age, yr	27.9 (21–44)	25.7 (19–41)	31.5 (20–45)	25.8 (20–35)
Height, cm	179 (163–193)	166 (159–175)	178 (158–191)	162 (155–167)
Weight, kg	85.0 (61–110)	60.9 (46–81)	81.4 (61–98)	57.9 (49–66)
BMI, kg/m^2	26.6 (19–36)	21.9 (23–31)	25.7 (22–32)	21.9 (20–25)

Values are means, with ranges in parentheses. BMI, Body mass index.

urine and water vapor (11, 30) as well as nonaqueous hydrogen exchange. ECW was quantified using the equations and techniques of Miller et al. (21), with the addition of a correction of 0.987 for the concentration of water in serum ultrafiltrate. The bromide concentration of plasma was assessed using Miller's anion-exchange high-pressure chromatography technique (20). Corrections were made for lost dosage of bromide in urine. Urine samples were cleaned using solid-phase cation-extraction filters (Sep Pak, Waters, Milford, MA), diluted threefold, and then analyzed using the same anion-exchange high-pressure chromatography technique used for blood. ICW was calculated as the difference between TBW and ECW.

BIS measurements. The proximal electrode placement technique was utilized (25), because the proximal placement is less sensitive to temperature and orthostatic effects (10). After the electrode site was cleaned with isopropyl alcohol, electrode patches (7.6 × 1.9 cm) with self-adhesive conducting gel (IS 4000, Xitron Technologies, San Diego, CA) were attached to the dorsal surface of the right foot and right hand for current injection. Detector electrodes were attached to the dorsal surface of the right leg with the proximal edge 1 cm distal to the center of the knee cap and on the dorsal surface of the right forearm with the proximal edge 1 cm distal to the midcrease of the antecubital fossa. BIS scans of the arm and leg segments were completed in triplicate using a Xitron Hydra 4200 BIS machine. Resistance and reactance were measured at 64 frequencies ranging from 5 kHz to 1 MHz, and the results were fit to a Cole-Cole curve using the vendor's curve-fitting algorithm. The output variables included resistance of extracellular fluid (R_e) and resistance of intracellular fluid (R_i) in ohms.

BIS equation development. The accuracy and precision of BIS were tested against tracer dilution during the ambulatory phase of *study 1* ($n = 28$) using previously published multifrequency BIS equations (11)

$$\text{ECW (kg)} = 0.11(\text{Ht}^2/R_e) + 3.0$$

$$\text{ICW (kg)} = 0.191(\text{Ht}^2/R_i) + 12.8$$

$$\text{TBW} = \text{ECW} + \text{ICW}$$

where Ht is height (in cm). As shown in Table 2, BIS-predicted TBW and ECW were not significantly different from tracer-dilution-measured volumes. However, there was a systematic 5% overestimation of ICW ($P = 0.02$). Thus a new set of predictive equations was developed through regression analysis using ambulatory phase data ($n = 28$) of *study 1*. First, Ht^2/R_e and Ht^2/R_i data were regressed against tracer-dilution-measured ECW and ICW, respectively. Outlying data points (residuals > 3 * SEE, where SEE is standard error of the estimate), including two subjects for ECW ($n = 26$) and one subject for ICW ($n = 27$), were removed from the data set. Then, new equations were developed for ECW and ICW

$$\text{ECW (kg)} = 0.104(\text{Ht}^2/R_e) + 4.09$$
$$(R^2 = 0.825, \text{SEE} = 1.22, P < 0.001)$$

$$\text{ICW (kg)} = 0.248(\text{Ht}^2/R_i) + 6.13$$
$$(R^2 = 0.898, \text{SEE} = 2.23, P < 0.001)$$

$$\text{TBW} = \text{ECW} + \text{ICW}$$

These equations were used to predict water volumes for the entire subject pool in *study 1* ($n = 28$) and *study 2* ($n = 18$).

Segmental BIS analysis. Segmental BIS was used to monitor changes in body water within regions of the body during 48 h of underfeeding and HDT of *study 2*. The segmental BIS procedure was similar to that used by Organ et al. (22) but with proximal (elbow-knee) electrode placement. Briefly, these measurements used a six-electrode technique with two current injection electrodes and two current detection electrodes in the standard right-side positions and two additional current detection electrodes on the left arm and leg. While the current injection leads remain fixed, the current detection leads can be moved so that scans can be made of the arm, the leg, and the trunk. Using this procedure and our proximal electrode placement, we measured BIS on three body segments: elbow to trunk ("upper arm"), trunk, and knee to trunk ("thigh"). Data produced from this analysis included segmental change in R_e and R_i and percent change in segmental R_e and R_i.

We combined segmental BIS data, dilution technique data (TBW, ECW/TBW, and ICW/TBW) from this study, and segmental body water estimations based on data from the cadaver study of Dempster and Gaughran (5) to estimate regional losses of ICW, ECW, and TBW (see APPENDIX). Cadaver segments included the arm (arm, forearm, hand), the leg (thigh, shank, foot), and the trunk (5). Cadaver segmental water content was estimated by *1*) calculating segmental density from measured mass and volume (see Tables 4 and 5 in Ref. 5), *2*) estimating segmental fat-free mass using a two-compartment model and densities of fat mass and fat-free mass compartments (13), and *3*) estimating segmental water content assuming a constant hydration of fat-free mass (13). Percentage of TBW for the segment was calculated by *1*) adding up all body segments to determine cadaver total body water, *2*) doubling segmental water for arm and leg segments, and *3*) dividing trunk, doubled arm, or doubled leg water by TBW and multiplying by 100 to determine %TBW of the segment. Error is present in these calculations from two major sources. The regional density of the fat-free mass compartment as well as the regional hydration of fat-free mass may vary from the whole body constants used (8). In addition, changes in the BIS R_e and R_i for the upper arm or thigh may not be exactly representative of changes in the entire arm or leg segments because of fluid shifts.

Table 2. *Accuracy and precision of BIS at 1 g during study 1 using previously published equations for BIS*

	ECW, kg ($n = 28$)		ICW, kg ($n = 28$)		TBW, kg ($n = 28$)	
	Absolute	Residual	Absolute	Residual	Absolute	Residual
Dil	15.5 ± 3.1		23.5 ± 6.7		39.0 ± 9.0	
BIS	15.1 ± 2.8	−0.5 ± 1.9	24.6 ± 6.7	1.1 ± 2.5	39.6 ± 9.4	0.6 ± 2.4
*P**	NS		0.02		NS	

Absolute values are means and between-subject SD. Residual values are means and SD for within-subject difference between bioelectrical impedance spectroscopy (BIS) and dilution. ECW, extracellular water; ICW, intracellular water; TBW, total body water. Equations for BIS are from Ref. 11. *Difference between methods; NS, not significant.

Nitrogen balance. Nitrogen balance during the 72 h of HDT of *study 2* was calculated from the difference of dietary protein intake and urinary nitrogen losses. Few subjects produced fecal samples during this period; thus no direct measurements of fecal nitrogen losses were made. Previous research suggests that fecal losses during the 72 h of HDT would be ~2.2 g nitrogen (13.5 g protein) for women and 2.9 g nitrogen (18 g protein) for men (19). These estimated fecal losses are not reflected in our calculated nitrogen and protein losses. Dietary protein intake for the defined hypocaloric diet was calculated using the Diet Planner program (University of California Clinical Research Center, San Francisco, CA). During hospitalization, all urine was stored under refrigeration in plastic collection jugs. Hydrochloric acid was added to the jugs to ensure retention of ammonia. Within 24 h of discharge, all samples were pooled, and an aliquot was taken for analysis. Urinary nitrogen was measured in quadruplicate using an automated nitrogen analyzer (Antek, Houston, TX).

Statistics. Descriptive data are presented as means ± SD. Statistical differences between before and after treatment were detected using paired *t*-tests. BIS equations were developed using simple linear regression techniques. Bland-Altman analysis (1) was used to test for potential relations between the mean difference and the average of techniques.

RESULTS

Estimation of water spaces by BIS during simulated microgravity and adequate nutrition. Table 3 shows the accuracy and precision of newly generated BIS equations at 4, 20, and 44 h of HDT during *study 1*. There were no significant differences between the dilution- and BIS-predicted ECW, ICW, and TBW volumes at any time point. Bland-Altman analysis showed no significant relation between the residuals and average of the techniques for ECW, ICW, and TBW (r^2 = 0.00–0.11) at any of the three time points. The accuracy of BIS for tracking within-subject change from baseline is shown in Fig. 1. There were no significant differences between the dilution- and BIS-predicted change in ECW, ICW, and TBW volumes at any measurement point.

Estimation of water spaces by BIS during simulated microgravity and suboptimal nutrition. The 72-h protocol produced an average of 2.1 kg weight loss, which is substantially more than that produced by short-term bed-rest protocols providing adequate nutrition (9). In addition, the nitrogen balance data suggest that subjects were substantially underfed during the protocol. During the 72 h of treatment, mean nitrogen losses were ~37 ± 8 $mg \cdot kg^{-1} \cdot day^{-1}$ (22 ± 8 g), which corresponds to a loss of ~135 ± 49 g protein. With the assumption that 1 g protein is associated with 4 g water, the loss of this body protein resulted in the loss of ~540 g water during the interval (12).

Figure 2 shows the accuracy and precision of newly generated BIS equations for predicting change in body water compartments during the 48 h of malnutrition and HDT of *study 2*. Dilution-measured ECW, ICW, and TBW significantly decreased during the treatment period, whereas only BIS-predicted ECW and TBW significantly decreased. Because whole body impedance methods are relatively insensitive to trunk water changes (2, 24), we hypothesized that BIS was insensitive to the changes in ICW during short-term malnutrition, because the major effect was localized to the trunk. To test this hypothesis, we examined segmental BIS data collected at the same time as the whole body scans. Using a combination of segmental BIS data, dilution-measured body water compartmentalization, and estimations of segmental water content from a cadaver study (5), we estimated regional losses of ICW, ECW, and TBW (Table 4; see APPENDIX). From this analysis, we estimated that although 65% of the ICW losses were localized in the trunk region, 74% of ECW losses also were localized in the trunk region.

DISCUSSION

The primary goal of this study was to determine the accuracy and precision of BIS for monitoring body fluid compartments under conditions of simulated microgravity. We completed two studies: one in which nutrition was adequate (*study 1*) and one in which subjects were severely underfed (*study 2*). We expected that HDT with nutritional adequacy would primarily result in losses of ECW (16). In contrast, we expected that the HDT and underfeeding protocol would cause loss of body protein and glycogen, which would cause additional losses of TBW from the ICW compartment.

As shown in Figs. 1 and 2, our protocol produced these desired effects. During *study 1*, subjects lost on

Table 3. *Accuracy and precision of BIS during simulated microgravity of study 1*

	ECW, kg (n = 28)		ICW, kg (n = 28)		TBW, kg (n = 28)	
	Absolute	Residual	Absolute	Residual	Absolute	Residual
4 h HDT						
Dilution	15.2 ± 3.1		23.3 ± 6.9		38.5 ± 9.2	
BIS	15.3 ± 2.6	0.1 ± 1.8	23.6 ± 6.3	0.4 ± 2.8	39.0 ± 8.8	0.5 ± 2.7
20 h HDT						
Dilution	14.9 ± 3.1		23.1 ± 6.6		38.0 ± 9.1	
BIS	15.0 ± 2.6	0.1 ± 1.5	23.3 ± 6.4	0.3 ± 2.8	38.4 ± 8.9	0.4 ± 2.4
44 h HDT						
Dilution	14.7 ± 3.2		22.9 ± 7.0		37.6 ± 9.4	
BIS	14.7 ± 2.7	0.0 ± 1.5	23.4 ± 6.4	0.5 ± 3.1	38.1 ± 9.0	0.6 ± 2.8

Absolute values are means and between-subject SD. Residual values are means and SD for within-subject difference between BIS and dilution. HDT, head-down tilt.

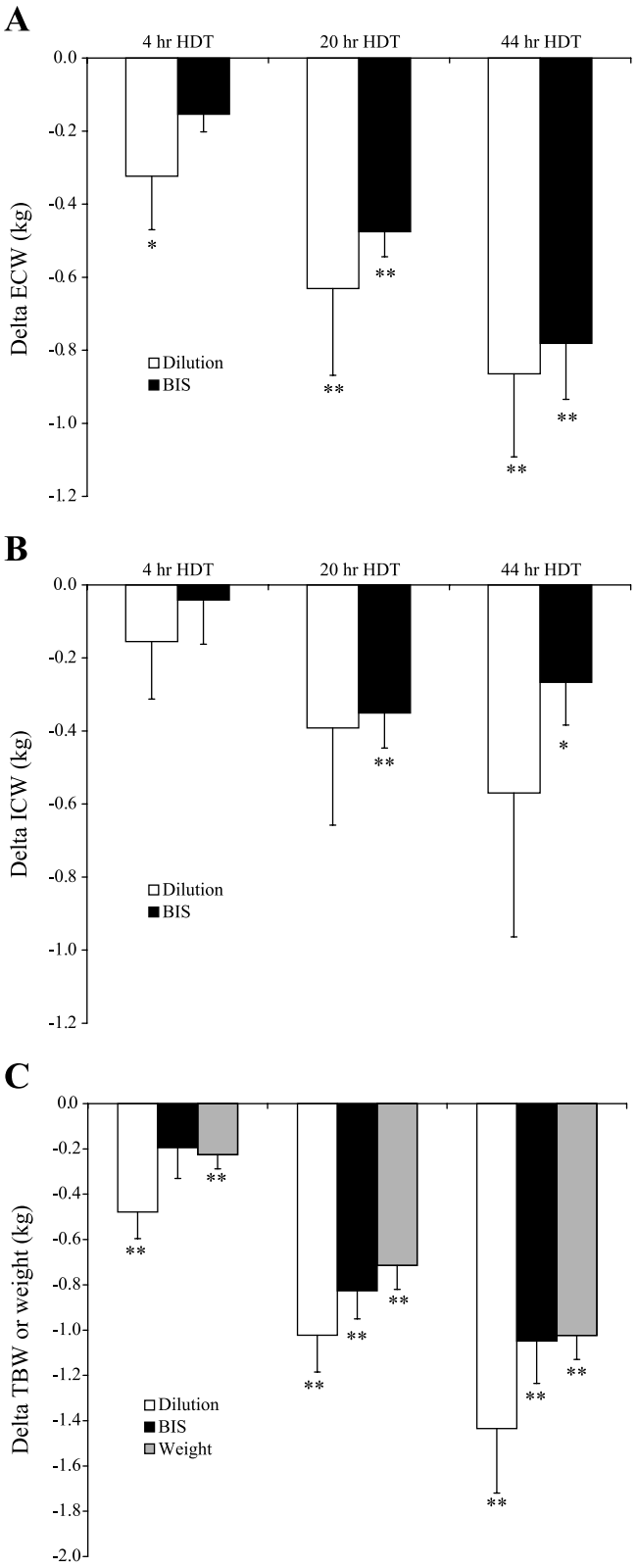

Fig. 1. Within-subject change in water compartments and weight from baseline (ambulatory) during 44 h of head-down tilt (HDT) in *study 1*. Changes in extracellular water (ECW, *A*) and intracellular water (ICW, *B*) were measured by dilution and predicted by bioelectrical impedance spectroscopy (BIS). *C*: change in total body water (TBW) measured by dilution and predicted by BIS, as well as weight change during the same time period. Values are means ± SE. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. baseline.

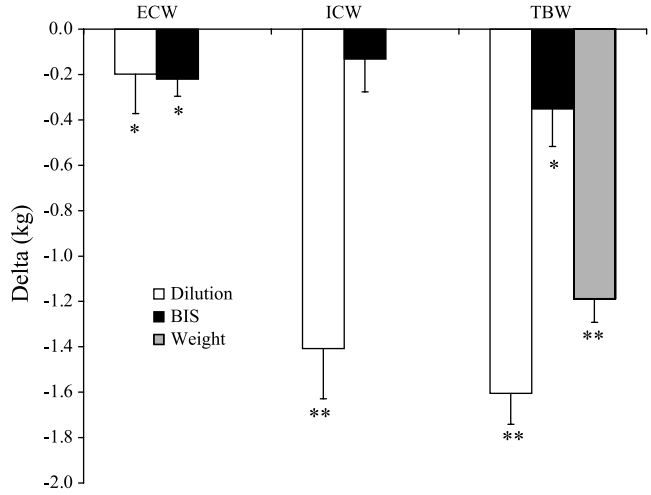

Fig. 2. Within-subject change in ECW, ICW, and TBW and weight during 48 h of HDT and undernutrition in *study 2*. Values are means ± SE. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. baseline.

average 1.0 kg body mass and 1.4 kg total body water during 44 h of HDT. According to dilution data, ~60% of body water was lost from the ECW compartment and 40% from the ICW compartment. In *study 2*, subjects lost on average 1.2 kg body mass and 1.6 kg TBW (by dilution) during the 48 h between measurement periods. Dilution data show that, of the body water lost, ~10% was ECW and 90% was ICW. Nitrogen balance data for the 72-h protocol provide additional evidence of malnutrition.

Study 1 demonstrated that when subjects are adequately fed and the proximal electrode placement technique is used, BIS calibration can be retained during simulated microgravity. BIS calibration was retained even though there were changes in the compartmentalization of fluids in the body, including ECW diuresis and headward fluid shifts. The ability of BIS to maintain this calibration was likely due to the use of the proximal electrode placement, in which detection electrodes are placed at the knee and elbow. This electrode position eliminates the calf from the path of the electrical current. Thus BIS is impervious to the shift of extracellular fluid out of the calf, a region of high sensitivity for BIS because of its small cross-sectional area.

Although the accuracy of BIS was excellent under these conditions, the precision was only modest. This imprecision can theoretically be due to instrument errors, within-subject changes in bioelectrical properties, imprecision in the dilution methods, or between-subject variations in geometry or bioelectrical properties. The latter two are important, because if they explain a significant amount of the variance in Table 3, then BIS can be used to track within-subject changes in water spaces with greater precision. Indeed, when we compare the within-subject changes in water spaces between the ambulatory phase and HDT, the variance is substantially reduced when within-individual changes are followed (Fig. 1). This shows that BIS can be used to track within-subject changes of biological

Table 4. *Segmental changes in resistances and water spaces during 48 h of HDT and undernutrition of study 2*

	%TBW*	$\%\Delta R_e$	ΔECW, kg	$\%\Delta R_i$	ΔICW, kg	ΔTBW, kg
Arms	12.7	2.8 ± 2.9	−0.05 ± 0.05	−0.6 ± 5.3	0.04 ± 0.17	−0.01 ± 0.16
Legs	36.0	1.8 ± 2.8	−0.09 ± 0.15	3.6 ± 3.2	−0.27 ± 0.28	−0.36 ± 0.35
Trunk	37.8	7.4 ± 5.3	−0.39 ± 0.31	5.7 ± 7.6	−0.42 ± 0.66	−0.81 ± 0.61
Total			−0.53 ± 0.46		−0.65 ± 0.78	−1.18 ± 0.72

Values are means ± SD; n = 18. %TBW was calculated using cadaver data in Dempster and Gaughran (5). Arms include both arms from shoulder to hand inclusive, legs include both legs from hip to feet inclusive, and trunk includes region from hip to shoulder. See APPENDIX for sample calculation of ΔECW and ΔICW. R_e and R_i, resistance of extra- and intracellular fluid, respectively.

relevance (500 g) with a high degree of precision as long as subjects are adequately nourished.

In *study 2*, where the changes in ICW were more substantial, the limitations of BIS for detecting short-term changes in compartmental water volume became apparent. As can be seen in Fig. 2, BIS accurately and precisely tracked the changes in ECW but far underestimated the changes in ICW. Thus losses in TBW also were greatly underestimated. The error in the prediction of TBW losses can be attributed to BIS, because the dilution and weight change data are in agreement.

Dilution data suggest that the dietary treatment was successful in reducing ICW and body cell mass, and segmental BIS analysis suggests that the effect was largely confined to the trunk. We presume that our estimated loss of trunk ICW is due to diuresis of glycogen- and protein-associated water in response to underfeeding. A loss in water weight of trunk organs (liver, heart, kidneys, spleen) has been documented in the early phases of acute starvation in rats (23, 27). Thus the combination of underfeeding and bed rest produced significant changes in trunk ICW, but the treatment was not of sufficient duration or intensity to produce limb ICW losses within the limits of detection by the BIS machine.

We hypothesized that BIS was insensitive to substantial changes in ICW, because the majority of ICW losses were in the trunk region, and whole body impedance methods are relatively insensitive to trunk changes (2, 24). Impedance methods are based on viewing the body as a series of five cylinders (2 arms, 2 legs, and 1 trunk), with each cylinder having a different cross-sectional area. Because the resistance of a cylinder is inversely proportional to its cross-sectional area, the resistance of the whole body is largely determined by the resistance of the limbs, which have small cross-sectional areas. It is estimated that an arm is ~4% of body weight and a leg is ~17% of body weight, but they contribute ~47% and 50%, respectively, of whole body resistance when electrodes were placed on the wrist and ankle (2). In our study, in which electrodes were placed on the knee and elbow, the upper arm accounted for 56% of total R_e and 59% of total R_i and the leg accounted for 34% of total R_e and 28% of total R_i. The trunk, which is ~46% of body weight (2), accounted for only 11% of total R_e and 12% of total R_i. Thus, even with large changes in trunk water volume, there is little if any influence on resistance at the whole body level because of the insensitivity of impedance methods to the trunk (24).

As the segmental BIS analysis showed (Table 4), the largest fractional changes in ICW did occur in the trunk. However, the largest fractional changes in ECW also occurred in the trunk region. Thus our hypothesis does not explain why trunk ICW changes were not detected and trunk ECW changes were detected. Recent data collected in our laboratory (unpublished observations) in an acute hypertonic dehydration protocol confirmed our observations in this study: BIS was able to detect acute ECW changes but was markedly insensitive to acute ICW changes. Previous validation studies of BIS for acute dehydration have utilized only small acute changes in ICW (11), so this problem has not been detected previously. We believe that the insensitivity of BIS to acute ICW changes may be due to a combined insensitivity of impedance techniques to trunk changes and a possible inability of BIS to measure ICW independently of ECW and TBW. Future microgravity validation studies of BIS should include a more gradual, longer term underfeeding protocol that is likely to produce whole body losses in ICW. In addition, future studies could incorporate new technology (e.g., magnetic resonance spectroscopy or combined Dixon/magnetic resonance imaging measurements) to directly measure limb water losses as a comparison for the BIS technique.

In conclusion, the BIS technique may have potential application for the measurement of nutritional status during spaceflight. The instrument has a volume of $<5{,}000$ cm^3, weighs <2 kg, and consumes little power. This study showed that the accuracy of BIS was not altered by simulated microgravity, as long as losses were predominantly from the ECW. This study also demonstrated that BIS can follow the changes in ECW and ICW that occur when a person moves from standing to HDT and that it can measure these changes with a relative precision (SD) of 2–4% in a single individual when subjects are adequately nourished. However, BIS was not effective at detecting a sudden decrease in trunk region ICW volume secondary to severe underfeeding. We suspect this is partially due to insensitivity of the whole body impedance technique in the trunk region, as well as a general inability to measure ICW independently of ECW and TBW.

APPENDIX

Segmental Calculations

Baseline dilution data. Baseline dilution data are as follows

$$ECW = 10.70 \text{ kg},$$

$$ICW = 16.09 \text{ kg},$$

$$TBW = 26.79 \text{ kg}$$

$$ECW/TBW = 10.70/26.79 = 0.399$$

$$ICW/TBW = 16.09/26.79 = 0.601$$

Segmental BIS data for arm. Baseline data are as follows

$$R_e = 174.29$$

$$R_i = 362.34$$

Posttreatment data are as follows

$$R_e = 181.07$$

$$R_i = 382.98$$

$$\text{change in } R_e = 6.78$$

$$\%\text{change in } R_e = 6.78/174.29 * 100 = 3.89$$

$$\text{change in } R_i = 20.64$$

$$\%\text{change in } R_i = 20.64/362.34 * 100 = 5.70$$

Change in water spaces. In the following equations, an opposite sign is added to the equation, because an increase in resistance corresponds to a decrease in water volume and vice versa. With the use of cadaver data from Dempster and Gaughran (5), it was estimated that 12.7% of TBW was found in the two arms (shoulder through hand)

$$\begin{aligned}\Delta ECW\ (g) &= (-) * (\%\text{change in } R_e/100) * \\ &\quad (\%TBW \text{ in upper arm}/100) * \\ &\quad TBW\ (kg) * ECW/TBW * 1{,}000\ g/kg \\ &= (-)\ (3.89/100) * (12.7/100) * (26.79) * \\ &\quad (0.399) * 1{,}000 \\ &= (-)\ 0.0389 * 0.127 * 26.79 * 0.399 * \\ &\quad 1{,}000 \\ &= -53\ g\end{aligned}$$

$$\begin{aligned}\Delta ICW\ (g) &= (-) * (\%\text{change in } R_i/100) * \\ &\quad (\%\ TBW \text{ in upper arm}/100) * \\ &\quad TBW\ (kg) * ICW/TBW * 1{,}000\ g/kg \\ &= (-)\ (5.70/100) * (12.7/100) * (26.79) * \\ &\quad (0.601) * 1{,}000 \\ &= (-)\ 0.0570 * 0.127 * 26.79 * \\ &\quad 0.601 * 1{,}000 \\ &= -117\ g\end{aligned}$$

$$\begin{aligned}\Delta TBW\ (g) &= \Delta ECW + \Delta ICW \\ &= -53 + -117 \\ &= -170\ g\end{aligned}$$

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